BACTERIOLOGICAL AND SEROLOGICAL EXAMINATION AND RISK FACTOR ANALYSIS OF SALMONELLA OCCURENCE IN SOW HERDS, INCLUDING RISK FACTORS FOR HIGH SALMONELLA SEROPREVALENCE IN RECEIVER FINISHING HERDS.

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Summary

A strong association between the seroprevalence in sows and the occurrence of *Salmonella typhimurium* among weaners has been shown. As shown several times for finisher herds, the risk-factors; ready-mixed pelleted feed and health status also apply to sow herds. Risk factors on the sow level, for high seroprevalence in finishers have been quantified. It has been shown, that isolating *Salmonella* in weaners is a risk factor for high seroprevalence in finishers. Feed factors; ready-mixed pelleted feed for both sows and finishers, dry feed for sows, have been shown to have a significant effect on high seroprevalence, monitored by meat juice samples at slaughter. The etiological fraction of ready-mixed pelleted feed for sows and for finishers is of the same magnitude, indicating that intervention on the sow level could prove to contribute considerably to the effect of intervention programs.

Keywords: Pig; feed; pelleted; monitoring; surveilance

Introduction

Since the Danish national *Salmonella enterica* surveillance programme (1) was established, the prevalence of seropositive slaughter pigs has decreased by approximately 50%, monitored by meat juice samples. In order to further reduce the occurrence of *Salmonella* in Danish pork, an investigation was carried out to illustrate the influence of the *Salmonella* status of sow-herds on the *Salmonella* infection in finisher-herds, to which the sow-herds deliver growers.

Materials and methods

Sixty-nine sow-herds were randomly selected from the Danish Central Holding Register. Inclusion criteria were a minimum of 100 sows and a maximum delivery of 100 finishers per year. From each herd, 20 non-stabilized blood samples were

drawn randomly from sows at different ages. Faecal pen samples were collected randomly from 20 pens, among weaners between 4 and 12 weeks of age. A questionnaire survey, with special emphasis on feeding and management, was performed, in order to identify potential risk-factors in the sow herds. Serological examination for specific antibodies to *Salmonella* was performed by means of an indirect enzyme immunosorbent assay, designated MIX-ELISA. The test includes the *Salmonella* LPS-antigens 1,4,5,6,7 and 12, representing approximately 90% of the *Salmonella* serotypes isolated in Danish pigs. Samples with a calibrated OD% above 40 were considered positive (2). Five grams of faeces from 5 different locations in each pen were pooled, and cultured by standard microbiological methods, including nonselective pre-enrichment followed by selective enrichment and serotyping.

All finishing herds receiving pigs (119) from the investigated sow herds were interviewed by telphone, and information about feeding and management procedures were obtained. For these 119 herds, results from the ongoing *Salmonella* surveillance program were obtained, covering the period from 3 months prior to visit of the sow herd, until 6 months after the visit.

Statistical methods: A logistic regression analysis was performed using the logistic procedure of the SAS System in order to identify risk factors for *Salmonella* occurrence among weaners in sow herds. The p-scale method was used to account for overdispersion in the data. Unit of observation was defined as the proportion of isolates per herd. Using the logistic regression model, individual potential risk-factors were investigated. If the p-value was less than 0.10, the risk-factor was included in the model for further analysis. Risk-factors with a p-value less than 0.05 in the final model, were evaluated as significant.

For the statistical analysis at the finisher-level, the logistic procedure of the SAS System was used but this time using Williams method to adjust for suspected overdispersion in the data. Unit of analysis was the individual pig, unit of observation was the herd. This means, that the relative risks are to be interpreted as the relative risk for one pig/meat-juice sample being positive, given the set of risk factors. Initially all potential risk factors were screened individually. Factors with a p-value less than 0.15 were used for further modelling. Known risk factors from other studies on finishers were forced into the model. In the final model, risk factors with a p-level below 0.05 were kept in the model. Feed factors for finishers were kept in the model, because other studies had shown, that they were risk factors. Two models were investigated, model 1 was a model, where the sow herd was characterised by the microbiological results from the sow herd, and for model 2, feed factors were used to describe the sow herd. Inclusion of both microbiological results and herd factors created multicollinearity problems, since feed factors for sow herds and microbiological results were highly correlated (table 3). Finally the aetiological fractions for ready-mixed pelleted feed for sows and finishers were calculated, using the results from the logistic regression model. The

calculation was performed by substituting use of ready-mixed pelleted feed by home-mixed feed for each herd, calculating the predicted value, given that the herd used home-mixed feed. Based on this predicted value, the number of positive pigs was calculated by multiplying by number of pigs per herd. Finally the etiological fraction was calculated using the following formula:

Etiological fraction=1-(predicted number of positive pigs after/predicted number of positive pigs before).

In these analyses, clustering was accounted for by adjusting for overdispersion at the finishing herd level. In reality this data-set represents a hierarchy. One sow herd delivers to between 1 and 4 finishing herds. Each herd is sampled on several dates, and each date includes several pigs. An alternative approach to these analyses could be to use random effect/multilevel models. We tried to use the the MlWiN package. We defined 4 levels in the model: Pig, sampledate, herd and sow herd. The effect of finishing herd factors were allowed to vary between clusters of sow herds. This creates 6 random factors: Sow-herd*feed source for finishers (ready-mixed pelleted vs. home-mixed feed), sow-herd*feed type for finishers (dry vs. liquid feed), sow herd, finishing herd, samplingdate and pig. A model including the factor finising-herdsize*sow herd could not converge. The 2.nd order PQL-estimation method was chosen for the MLWiN-model.

Results

Salmonella was isolated from pen samples in 13 (19%) sow herds. With the exception of weaners in the age group of four weeks, *Salmonella* was isolated to the same extent in all age groups. Table 1 shows the distribution of positive pen samples, among positive herds, and it shows that a sample size of a minimum of 20 is recommended to give sufficient confidence to find at least one positive pen sample, if *Salmonella* is present in the herd.
Fifty-three of 1371 (3.9%) sows were seropositive using the mixed-ELISA with a cut-off of 40 OD%. Seropositive sows were evenly distributed according to parity. Comparing of microbiological and serological results, table 2 shows a strong association between a seroprevalence >10% in sows and occurrence of *Salmonella typhimurium* among weaners. A similar association between other *Salmonella* serotypes among weaners and serology could not be demonstrated.
Among those potential risk factors provided by the questionnaire, ready-mixed pelleted feeding to sows seems to be associated with a higher risk of detecting the presence of *Salmonella* among weaners. It was not possible, to conclude if type of feed source (ready-mixed pelleted vs. home-mixed feed) was a risk factor for weaners. Table 3 shows selected results from the questionnaire.

It was not possible to measure the effect of liquid feeding in this investigation, as only 6 herds used a liquid feeding system and *Salmonella* was not detected in any of these. However, an effect of herd health status was shown. *Salmonella* was more likely to be found in MS-herds than in conventional herds. Table 4 shows the results of the logistic regression analysis, with feeding source and health status as explanatory variables and the proportion of positive pen samples as the dependent variable. On the finishing level, results from three different models are presented in table 5. Isolating *Salmonella* from from weaners increases the risk 3 times for seropositivity in finishers. Use of ready-mixed pelleted feed for sows is associated with a relative risk of app. 2.5 for seropositivity in the finishers, compared to use of home-mixed meal for sows. In these analyses we could demonstrate that use of liquid feed for sows is a protective factor for seropositivity in finishing pigs, although it could not be demonstrated in the first part of the study. Use of ready-mixed feed for finishers is a risk factor for seropositivity in finishers, and doubling herd size is also associated with an increase in risk.
In table 6 are shown the etiological fractions for ready-mixed pelleted feed for sows and finishers alone and combined, using the estimates from model 2. The effect of introducing home-mixed feed in all sow herds, finishing herds and for both herd types on number of positive pigs in the population is also shown.

Discussion

There is a strong association between the seroprevalence in sows and the occurrence of *Salmonella typhimurium* among weaners. As shown several times for finisher herds, the risk-factors; purchased feeding and health status also apply to sow herds. Our study could not demonstrate a protective effect of wet feeding, as only 6 herds used wet-feeding. However they were all negative for *Salmonella* among weaners, so it seems reasonable to assume that wet-feeding may have a protective effect in sow herds, as seen in finisher herds.
To our knowledge, this is the first time, sow level risk factors for high seroprevalence in finishers have been quantified. Previous studies have given conflicting evidence, ranging from no influence to some influence. We found, that isolating *Salmonella* in weaners is a risk factor for high seroprevalence in finishers, and we found that the feed factors found in previous studies on finishers, were important also on the sow-level. The etiological fraction of ready-mixed pelleted feed for sows and for finishers is of the same magnitude, indicating that intervention on the sow level could prove to contribute considerably to the effect of intervention programs. In the Danish *Salmonella* Reduction programme, focus has been on finishing herds, and the overall seroprevalence has been reduced from 5.5 % to 2.4-3 % over the last 4 years. Improving the results further will probably only be possible, if the reduction programme is extended to the sow herds.

Using multi-level modelling provided results that were comparable to the results found in the simple logistic model, except for the effect of herd-size and use of liquid feed for finishers.
We were not able to demonstrate a significant protective effect of liquid feed for finishers found in other studies (3). This is probably due to the fact, that there were only 19 finisher herds using liquid feed, and a large proportion of these were using home-mixed feed for finishers.

References

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Table 1. Number of positive pen samples, when 20 samples per herd are taken, in positive herds.

Number of isolates	1	2	3	4	6	8	9
Number of herds	3	3	2	1	1	2	1
Of these S. typhimurium	0	1	1	1	0	2	1

Table 2. Distribution of serological findings in sows compared to microbiological findings among weaners.

	Salm. typhimurium	Other serotypes	Negative	Total
Seroprevalence > 10 %	6	0	3	9
Seroprevalence < 10%	0	7	53	60
Total	6	7	56	69

Table 3. Distribution of feeding system to sows compared to microbiological findings among weaners.

	Salmonella +	Salmonella -	Total
Ready-mixed pelleted feed	12	24	36
Home-mixed feed	1	32	33
Total	13	56	69

Table 4. Risk-factors for positive pen samples among weaners in sow herds.

Risk factor	Approx. Relative risk	Confidence interval	P-value
Ready-mixed pelleted feed	25	3.87-16761	<0.0001
Home-mixed feeding	1		
Conventional herd	17	1.5-25000	0.05
MS-herd	10	0.67-15000	
SPF-herd	1		

Table 5. Relative risk for seropositivity in finishers.

Risk factor	Model 1 OR(C.I.)	Model 2 OR(C.I.)	MlWiN OR(C.I.)
Microbiol. results from sowherd:	3.04 (1.01-7.85)		
Salmonella Typhimurium	3.19 (1.27-7.47)		
Other serotypes	1		
Negative			
Ready-mixed pelleted feed for sows		2.44 (1.08-6.16)	2.37 (1.09-5.25)
Home-mixed meal for sows		1	1
Dry feed for sows		9.18 (1.25-893)	11.20 (2.19-57.4)
Liquid feed for sows		1	1
Ready-mixed pelleted feed for fin.	3.12 (1.18-9.91)	2.86 (1.12-8.52)	4.16 (1.74-9.97)
Home-mixed meal for finishers	1	1	1
Dry feed for finishers	2.33 (0.66-11.5)	2.03 (0.64-8.75)	1.08 (0.52-9.63)
Liquid feed for finishers	1	1	1
Doubling herd size	1.27 (1-1.69)	1.30 (1.03-1.74)	1.17 (0.93-1.48)

Table 6. Etiological fractions for ready-mixed pelleted feed, and effect of changing to home-mixed feed on seroprevalence.

Risk factor changed	Etiological fraction	Predicted prevalence after change
No change	-	3.9 %
Change sow feed	0.46	2.1 %
Change finisher feed	0.46	2.1 %
Change both	0.71	1.1 %